

IN THE SPECIFICATION:

◊ Please amend the first complete paragraph on page 1 as follows:

This application is a continuation of U.S. Ser. No. 09/302,629, filed April 30, 1999 (U.S. Pat. No. 6,316,418), which is a continuation of U.S. Ser. No. 09/087,811, filed May 29, 1998 (U.S. Pat. No. 6,054,436), which is a continuation of U.S. Ser. No. 08/292,597, filed Aug. 18, 1994 (U.S. Pat. No. 5,834,266), which is a continuation-in-part of U.S. Ser. No. 08/179,143 filed Jan. 7, 1994 (abandoned), which is a continuation-in-part of U.S. Ser. No. 08/093,499 filed Jul. 16, 1993 (abandoned); U.S. Ser. No. 09/302,629 is also a continuation-in-part of U.S. Ser. No. 08/196,043 filed Feb. 11, 1994 (abandoned), which is a continuation-in-part of U.S. Ser. No. 08/179,748 filed Jan. 7, 1994 (abandoned), which is a continuation-in-part of U.S. Ser. No. 08/092,977 filed Jul. 16, 1993 (abandoned), which is a continuation-in-part of U.S. Ser. No. 08/017,931 filed Feb. 12, 1993 (abandoned). ~~The contents of each of these applications is hereby incorporated by reference into the present disclosure.~~

◊ Please amend the description of Figure 4 found on page 13, lines 29-30, as follows:

Figs. 4A, 4B and 4C are sequences of the primers used in the constructions of the plasmids employed in the subject invention (SEQ ID NOs: 4-6, 8-10, 12, 14-35, 37-40).

◊ Please amend the description of Figure 6 found on page 13, lines 33-35, as follows:

Fig. 6A and 6B is a chart of the activity of various ligands with the TAg Jurkat cells described in Example 1. For Fig. 6B, see also Spencer et al, Science 262,1019, Fig. 3 and caption, esp. 3B on p. 1020 therein.

◊ Please amend the description of Figure 16 found on page 14, lines 18-19, as follows:

Fig. 16A and 16B depicts synthetic schemes for HED and HOD reagents based on FK506-type moieties.

◊ Please amend the description of Figure 18 found on page 14, line 21, as follows:

Fig. 18A and 18B is an overview of the fusion cDNA construct and protein MZF3E.

◊ Please amend the paragraph bridging pages 30-31 as follows:

For the most part, for ease of construction, the transmembrane domain of the cytoplasmic domain or the receptor domain can be employed, which may tend to simplify the construction of the fused protein. However, for the lipid membrane retention domain, the processing signal will usually be added at the 5' end of the coding sequence for N-terminal binding to the membrane and, proximal to the 3' end for C-terminal binding. The lipid membrane retention domain will have a lipid of from about 12 to 24 carbon atoms, particularly 14 carbon atoms, more particularly myristoyl, joined to glycine. The signal sequence for the lipid binding domain is an N-terminal sequence and can be varied widely, usually having glycine at residue 2 and lysine or arginine at residue 7 (Kaplan, et al., Mol. Cell. Biol. (1988) 8, 2435). Peptide sequences involving post-translational processing to provide for lipid membrane binding are described by Carr, et al., PNAS USA (1988) 79, 6128; Aitken, et al., FEBS Lett. (1982) 150, 314; Henderson, et al., PNAS USA (1983) 80, 319; Schulz, et al., Virology (1984), 123, 2131; Dellman, et al., Nature (1985) 314, 374; and reviewed in Ann. Rev. of Biochem. (1988) 57, 69. An amino acid sequence of interest includes the sequence M-G-S-S-K-S-K-P-K-D-P-S-Q-R (SEQ ID NO: 1). Various DNA sequences can be used to encode such sequence in the fused receptor protein.

◊ Please amend the 3rd complete paragraph on page 50 as follows:

A fourth example involves treatment of chronic pain with endorphin via encapsulation. A stock of human fibroblasts is transfected with a construct in which the chimeric transcriptional regulatory protein controls the transcription of human endorphin. The DNA construct consists of three copies of the binding site for the HNF-1* transcription factor GTTAAGTTAAC (SEQ ID NO: 2), upstream of a TATAAA site and a transcriptional initiation site. The endorphin cDNA would be inserted downstream of the initiation site and upstream of a polyadenylation and termination sequences. Optionally, the endorphin cDNA is outfitted with "PEST" sequences to make the protein unstable or AUUA sequences in the 3' nontranslated region of the mRNA to allow it to be degraded quickly.

◊ Please amend the 1st complete paragraph on page 57 as follows:

The plasmid pSXNeo/IL2 (IL2-SX) (Fig. 1), which contains the placental secreted alkaline phosphatase gene under the control of human IL-2 promoter (-325 to +47; MCB(86) 6,

3042), and related plasmid variants (i.e. NFAT-SX, NF B-SX, OAP/Oct1-SX, and AP-1-SX) in which the reporter gene is under the transcriptional control of the minimal IL-2 promoter (-325 to -294 and -72 to -47) combined with synthetic oligomers containing various promoter elements (i.e. NFAT, NK B, OAP/Oct-1, and AP1, respectively), were made by three piece ligations of 1) pPL/SEAP (Berger, et al., Gene (1988) 66,1) cut with SspI and HindIII; 2) pSV2/Neo (Southern and Berg, J. Mol. Appl. Genet. (1982) 1, 332) cut with NdeI, blunted with Klenow, then cut with Pvul; and 3) various promoter-containing plasmids (i.e. NFAT-CD8, B-CD8, cx121acZ-Oct-1, AP1-LUCIF3H, or cx151L2) described below) cut with PvuI and HindIII, NFAT-CD8 contains 3 copies of the NFAT-binding site (-286 to 31 257; Genes and Dev. (1990) 4, 1823) and cx121acZ-Oct contains 4 copies of the OAP/Oct-1(ARRE-1) binding site (MCB, (1988) 8, 1715) from the human IL-2 enhancer; B-CD8 contains 3 copies of the NFB binding site from the murine light chain (EMBO (1990) 9, 4425) and AP1-LUCIF3H contains 5 copies of the AP-1 site (5'-TGA-CTCAGCGC-3' [SEQ ID NO:3]) from the metallothionein promoter:

◊ Please amend the 1st complete paragraph on page 64 as follows:

To obtain the binding domain for FK506, plasmid rhEKBP (provided by S. Schreiber, Nature (1990) 346, 674) was used with P#6052 (SEQ ID NO:33) and P#6053 (SEQ ID NO:35) to obtain a 340 bp XhoI-SalI fragment containing human FKBP12. This fragment was inserted into pBluescript digested with XhoI and SalI to provide plasmid FK12/KS, which was the source for the FKBP12 binding domain. SPZ/KS was digested with XhoI, phosphatased (cell intestinal alkaline phosphatase; CIP) to prevent self-annealing, and combined with a 10-fold molar excess of the XhoI-SalI FKBP12-containing fragment from FK12/KS. Clones were isolated that contained monomers, dimers, and trimers of FKBP12 in the correct orientation. The clones 1FK1/KS, 1FK2/KS, and 1FK3/KS are comprised of in the direction of transcription; the signal from the murine MHC class II gene I-E, a monomer, dimer or trimer, respectively, of human FKBP12, and the transmembrane and cytoplasmic portions of CD3. Lastly, the SacII-EcoRI fragments were excised from pBluescript using restriction enzymes and ligated into the polylinker of pBJ5 digested with SacII and EcoRI to create plasmids 1FK1/pBJ5, 1FK2/pBJ5, and 1FK3/pBJ5, respectively. See Figs. 3 and 4 (SEQ ID NOs: 4-6, 8-10, 12, 14-35, 37-40).

◊ Please amend the paragraph bridging pages 64-65 as follows:

A myristoylation sequence from c-src was obtained from Pellman, et al., Nature 314, 374, and joined to a complementary sequence of CD3 to provide a primer which was complementary to a sequence 3' of the transmembrane domain namely P#8908 (SEQ ID NO: 23). This primer has a SacII site adjacent to the 5' terminus and a XhoI sequence adjacent to the 3' terminus of the myristoylation sequence. The other primer P#8462 (SEQ ID NO:21) has a SalI recognition site 3' of the sequence complementary to the 3' terminus of CD3, a stop codon and an EcoRI recognition site. Using PCR, a 450 bp SacII-EcoRI fragment was obtained, which was comprised of the myristoylation sequence and the CD3 sequence fused in the 5' to 3' direction. This fragment was ligated into SacII/EcoRI-digested pBJ5(XhoI)(SalI) and cloned, resulting in plasmid MZ/pBJ5. Lastly, MZ/pBJ5 was digested with SalI, phosphatased, and combined with a 10-fold molar excess of the XhoI-SalI FKBP12-containing fragment from FK12/KS and ligated. After cloning, the plasmids comprising the desired constructs having the myristoylation sequence, CD3 and FKBP12 multimers in the 5'-3'- direction were isolated and verified as having the correct structure. See Figs. 2 and 4 (SEQ ID NOs: 4-6, 8-10, 12, 14-35, 37-40).

◊ Please amend the diagram at the bottom of page 65 as follows:

5' end of PCR amplified product:

-----GAL4(I-147)----->
Sac II M K L L S S I (SEQ ID NO: 44)
5' CGACACCGCGGCCACCATGAAGCTACTGTCTTCTATCG (SEQ ID NO: 41)
Kozak

◊ Please amend the diagram at the top of page 66 as follows:

3' end of PCR amplified product:

<----GAL4 (1-147----) |
 R Q L T V S (SEQ ID NO: 46)
 5' GACAGTTGACTGTATCGGTCGACTGTCG (SEQ ID NO: 45)
 3' CTGTCAACTGACATAGCCAGCTGACAGC (SEQ ID NO: 77)
 SalI

◊ Please amend the diagram at the bottom of page 66 as follows:

5' end of PCR amplified product:

SacII | --HNF1(1-281)--->
 M V S K L S (SEQ ID NO: 50)
 5' CGACACCGCGGCCACCATGGTTCTAAGCTGAGC (SEQ ID NO: 49)
 Kozak

3' end of PCR amplified product:

<<---- HNF1 (1-282) ----|
 A F R H K L (SEQ_ID NO: 52)
 5 ' CCTTCGGCACAAAGTTGGTCGACTGTCG (SEQ_ID NO: 51)
 3 ' GGAAGGCCGTGTTCAACCAGCTGACAGC (SEQ_ID NO: 78)
 SalI

◊ Please amend the diagram in the middle of page 67 as follows:

Insertion of generic start site.

Kozak	
	M L E <u>(SEQ ID NO: 54)</u>
5'	GGCCACCATGC <u>(SEQ ID NO: 53)</u>
3'	CGCCGGTGGTACGAGCT <u>(SEQ ID NO: 79)</u>
SacII	XhoI
overhang	overhang

◊ Please amend the diagram at the top of page 68 as follows:

Insertion of NLS into generic start site

◊ Please amend the diagram at the bottom of page 68 as follows:

5' end of PCR amplified product:

Salli | --VP16(413-490)--->
A P P T D V (SEQ_ID_NO: 64)
5' CGACAGTCGACGCCCGGACCGATGTC (SEQ_ID_NO: 61)

◊ Please amend the diagram at the top of page 69 as follows:

3' end of PCR amplified product:

<-- VP16(413-490) --> |
D E Y G G (SEQ ID NO: 66)
5' GACCGAGTACGGTGGGCTCGAGTGTG (SEQ ID NO: 65)
3' CTGCTCATGCCACCCGAGCTCACAGC (SEQ ID NO: 81)
Xho1

◊ Please amend page 69, lines 11-24, as follows:

Oligonucleotides:

#37 38 mer/0.2 um/Off 5'CGACACCGCGGCCACCATGAAGCTACTGTCTTCTATCG (SEQ ID NO:41)

#38 28 mer/0.2 um/Off 5'CGACAGTCGACCGATACTGTC (SEQ ID NO:42)

#39 34 mer/0.2 um/Off 5'CGACACCGCGGCCACCATGGTTCTAAGCTGAGC (SEQ ID NO:49)

#40 28 mer/0.2 um/Off 5'CGACAGTCGACCAACTTGTGCCGGAGG (SEQ ID NO:48)

#43 29 mer/0.2 um/Off 5'CGACAGTCGACGCCCCCCCCGACCGATGTC (SEQ ID NO:61)

#44 26 mer/0.2 um/Off 5'CGACACTCGAGCCCACCGTACTCGTC (SEQ ID NO:62)

#45 26 mer/0.2 um/Off 5'GGCCACCATGC (SEQ ID NO:53)

#46 18 mer/0.2 um/Off 5'TCGAGCATGGTGGCCGC (SEQ ID NO:55)

#47 27 mer/0.2 um/Off 5'TCGACCCCTAAGA-(C/A)-GAAGAGAAAGGTAC (SEQ ID NO:56)

#48 27 mer/0.2 um/Off 5'TCGAGTACCTTCTCTTC-(G/T)-TCTTAGGG (SEQ ID NO:57)

◊Please amend the paragraph bridging pages 88-89 as follows:

3. Mutant hFKBP12 cDNA libraries hFKBP12 may be digested with EcoRI and HindIII, blunted and cloned into pAS1 (Durfee et al, *supra*) that has been cut with NcoI and blunted. This plasmid is further digested with NdeI to eliminate the NdeI fragment between the NdeI site in the polylinker sequence of pAS1 and the 5' end of hFKBP12 and religated. This generated the hFKBP12-GAL4 DNA binding domain protein fusion. hFKBP was reamplified with primers #11206 and #11210, Primer Table (SEQ ID NOs: 67-76):

IN THE CLAIMS:

Please cancel, without prejudice, claims 1-10 and 12-35 which were previously withdrawn from consideration as directed to a nonelected invention.

1-10. (Cancelled)

11. **(Previously presented)** A recombinant chimeric protein comprising at least one ligand-binding domain which binds to a selected ligand and an action domain which is heterologous with respect to the ligand-binding domain, wherein the selected ligand binds to the ligand-binding domain and to a ligand-binding domain of another protein to form a ligand cross-linked complex with the two protein molecules, and further has one or more of the following characteristics:

- (i) the ligand is not a protein;
- (ii) the ligand has a molecular weight less than 5 kD; and
- (iii) the ligand is membrane permeable; and

wherein the action domain induces apoptosis in a cell following formation of the ligand cross-linked complex.

12-35. (Cancelled)

36. **(Previously presented)** The chimeric protein of claim 11, wherein the chimeric protein comprises two or more ligand-binding domains.

37. **(Previously presented)** The chimeric protein of claim 11, wherein the chimeric protein comprises at least one ligand-binding domain which binds to a ligand having a molecular weight less than 3 kD.

38. **(Previously presented)** The chimeric protein of claim 11, wherein the chimeric protein comprises at least one ligand-binding domain having between 50 and 350 amino acid residues.

39. **(Previously presented)** The chimeric protein of claim 11, wherein the chimeric protein

comprises at least one naturally-occurring ligand binding domain.

40. **(Previously presented)** The chimeric protein of claim 11, wherein the chimeric protein comprises at least one ligand-binding domain comprising a non-naturally-occurring peptide sequence.

41. **(Previously presented)** The chimeric protein of claim 11, wherein the chimeric protein binds to the selected ligand with a k_D value less than or equal to about 10^{-6} M.

42. **(Previously presented)** The chimeric protein of claim 11, wherein the chimeric protein comprises at least one ligand binding domain comprising an immunophilin domain.

43. **(Previously presented)** The chimeric protein of claim 11, wherein the chimeric protein comprises at least one ligand-binding domain which binds to FK506, FK520, or derivatives thereof.

44. **(Previously presented)** The chimeric protein of claim 11, wherein the action domain comprises a cytoplasmic portion of a Fas or TNF receptor sufficient to induce apoptosis in a cell following formation of the ligand cross-linked complex.

45. **(Previously presented)** The chimeric protein of claim 11, wherein the action domain comprises a cytoplasmic domain of a receptor which induces apoptosis.

46. **(Previously presented)** The chimeric protein of claim 11, wherein said ligand is a synthetic organic molecule having a molecular weight of less than 5 kDa.